JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1989, p. 2328–2330
0095-1137/89/102328-03$02.00/0

Vol. 27, No. 10

Lactobacillus acidophilus Inhibits Growth of *Campylobacter pylori* In Vitro

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Received 21 March 1989/Accepted 13 July 1989

***Campylobacter pylori* has been implicated as a causative factor in acid-peptic disease. *Lactobacillus acidophilus* is known to inhibit the growth of pathogens in the human gastrointestinal tract. We recovered *C. pylori* from gastric antral biopsies of seven patients with acid-peptic disease; the isolates were then cultured in brucella broth. The effect of *L. acidophilus* (cultured in DeMan-Rogosa-Sharpe broth) on the growth of *C. pylori* was tested by a mixed culture technique. *L. acidophilus* inhibited the growth of all seven isolates of *C. pylori* in vitro. All these isolates were also inhibited by the *L. acidophilus* culture supernatant (brucella blood agar cup technique) obtained at or after 48 h of incubation. Inhibition of *C. pylori* growth was also observed with 1 and 3% lactic acid but not with 0.5 and 1% hydrogen peroxide, the *L. acidophilus* sonic extract, or a citrate-phosphate buffer (pH 4.0). We conclude that the inhibitory action of *L. acidophilus* on *C. pylori* is dependent on an extracellular secretory product, probably lactic acid. This inhibitory effect may be of therapeutic relevance in patients with *C. pylori*-positive acid-peptic disease.**

Campylobacter pylori has been recently implicated as an important etiologic factor in antral gastritis associated with peptic ulcer disease (14). Drugs which inhibit the growth of this organism are known to reduce the risk of recurrence of peptic ulcer (12).

Lactobacillus acidophilus is a commensal in the human alimentary tract, its concentration in the normal stomach being 0 to 10^3/ml (4). Being acid resistant, it persists in the stomach longer than other bacteria do (5). Oral administration of *L. acidophilus* has also been found to be useful in various conditions associated with altered intestinal flora, e.g., traveler's diarrhea (20) and antibiotic-associated colitis (7). Its beneficial effect may be related to its ability to suppress the growth of pathogens, probably by the secretion of antibacterial substances including lactic acid (6), hydrogen peroxide (1), and various antibiotics (6, 19; C. Deneke, M. Silva, and N. Jacobus, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, O-29, p. 266).

We undertook this in vitro study to evaluate the effects of *L. acidophilus* and its metabolites on *C. pylori* growth as a preliminary to its clinical evaluation in acid-peptic disease. The effect of a buffer solution, with a pH corresponding to that of *L. acidophilus* supernatant, was also evaluated.

MATERIALS AND METHODS

Antral biopsies obtained through a flexible gastroduodenoscope from patients with acid-peptic disease were transported in 1 ml of nutrient broth and inoculated directly onto brucella blood agar supplemented with 6 mg of vancomycin and 20 mg of nalidixic acid per liter. The plates were incubated at 37°C in a microaerophilic environment in a candle jar for 3 to 5 days. *C. pylori* was identified by Gram staining; presence of catalase (10), oxidase (13), and urease (10); and absence of sodium hippurate hydrolysis (7).

L. acidophilus was obtained from commercially available lyophilized cultures (Lactisyn; Franco Indian Pharmaceuticals, Bombay, India). The organism was grown in DeMan-Rogosa-Sharpe (MRS) broth (2) in a microaerophilic environment. All the media were obtained from HiMedia Laboratories Pvt. Ltd., Bombay, India.

Effect of *L. acidophilus* on *C. pylori* growth: mixed culture technique (6). *L. acidophilus* was incubated in 5 ml of MRS broth (pH 6.5) and *C. pylori* was incubated in 5 ml of brucella broth (pH 7.2), both for 48 h in a microaerophilic environment. The optical density of the culture was then adjusted at 420 nm to that of McFarland tube no. 1 (3×10^8 cells per ml) (21).

A 1-ml portion of broth taken from each of the tubes was mixed and incubated in a microaerophilic environment. Samples from the mixed culture were streaked at 0, 8, 24, and 48 h on brucella blood agar and MRS agar. The plates were incubated in a microaerophilic environment for 48 h, and the CFU were counted by the spread-plate technique (15).

Effects of supernatant and sonic extract of *L. acidophilus* on *C. pylori* growth: agar cup method. A 1-ml portion of *C. pylori* grown in brucella broth was mixed with 18 ml of brucella blood agar. Ten-millimeter cups were then prepared with a cork borer. A 5-ml portion of MRS broth with lactobacilli was sonicated in an ultrasonic processor for 15 min at 6 mHz. The sample was centrifuged, and 0.1 ml of the sonic extract was used for the study. Portions, 0.1 ml each, of *L. acidophilus* supernatant obtained at 48, 72, and 96 h of incubation (pH 4.0 in each) and 0.1 ml of the sonic extract were added to separate cups. The plates were incubated in a microaerophilic environment for 48 h.

Effects of lactic acid, hydrogen peroxide, and buffer on *C. pylori* growth. The effects of 1 and 3% lactic acid (pH 2.0), 0.5 and 1% hydrogen peroxide (pH 6.5), and a citrate-phosphate buffer (pH 4.0) (3) on *C. pylori* growth were tested by the agar cup method, as described above.

Statistical analysis was done by the Wilcoxon signed rank test (16).

RESULTS

Seven isolates of *C. pylori* were recovered.

At the end of 48 h of incubation, the pH in the MRS broth containing the *L. acidophilus* culture dropped from 6.5 to

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4.0; in the brucella broth containing the *C. pylori* culture, the pH persisted at 7.2. In the mixed culture study, the pH of the mixed broth remained consistently around 6.5 during the 48-h incubation period. Plates streaked with the mixed culture at 0 and 8 h of incubation showed good growth (10^8 CFU/ml) of *C. pylori* as well as of *L. acidophilus*. The plates streaked after 24 h of incubation of the mixed culture showed a decrease in the colony count of *C. pylori* to a median of 10^2 CFU/ml ($P < 0.02$) (range, 0 to 55×10^2 CFU/ml) at 24 h, and those streaked after 48 h of incubation of the mixed culture showed no growth ($P < 0.02$) of *C. pylori* in MRS broth or on brucella blood agar at the end of 5 days. In both the cases, *L. acidophilus* continued to grow.

By the agar cup method, *L. acidophilus* supernatant obtained at 48 h of incubation inhibited *C. pylori* growth to a median of 18 mm (range, 10 to 24 mm); supernatant obtained at 72 h inhibited *C. pylori* to a median of 26 mm (range, 21 to 34 mm), and that at 96 h showed 25 mm (range, 18 to 36 mm) of inhibition. No inhibition was obtained with the sonic extract.

Lactic acid at concentrations of 1 and 3% inhibited *C. pylori* growth to medians of 18 mm (range, 15 to 25 mm) and 24 mm (range, 17 to 30 mm), respectively. No inhibition was obtained with 0.5 and 1% hydrogen peroxide or with the citrate-phosphate buffer.

DISCUSSION

We observed a significant suppression in the growth of *C. pylori* in the presence of *L. acidophilus*. This occurred only when the two organisms were incubated together for more than 24 h. A similar suppression was obtained with the supernatant from the *L. acidophilus* culture broth, as well as with 1 and 3% lactic acid. However, no suppression was observed with the sonic extract, hydrogen peroxide, or a buffer solution with a pH corresponding to that of the supernatant.

Lactobacilli belong to the group of lactic acid bacteria. Glucose fermentation of these organisms produces at least 50% of the end product carbon as lactic acid (18). *L. acidophilus* thrives best in an acid environment; hence its ability to persist longer in the stomach than any other bacteria (5). However, this organism survives even at a higher pH and has been shown to suppress pathogens by overgrowth in the human intestinal tract (11). This forms the basis for its use in the treatment of conditions associated with intestinal bacterial overgrowth.

C. pylori has been implicated in the gastric mucosal damage associated with acid-peptic disease. The organism has a pH tolerance in vitro between 6 and 8 (9). This is enhanced to 2 or 1.5 in the presence of urea. The pH (6.5) in the mixed culture broth that we used would normally permit the growth of *C. pylori*. The suppression that we observed is thus likely to be due to the presence of *L. acidophilus*.

The major groups of inhibitory compounds produced by lactic acid bacteria are lactic and volatile acids (6), hydrogen peroxide (1), and antibioticlike compounds (acidophilins or bacteriocins) (6, 19; Deneke et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). The spectrum of the latter is still being unraveled. While lactic acid is the major catabolic product, it may not be the most inhibitory. The concentrations of lactic acid that we found to be inhibitory are within the levels reported to be produced by *L. acidophilus* (17). In another study (H. C. Wong and Y. L. Chen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, P-24, p. 278), 0.1 M lactate was shown to be inhibitory to *Bacillus cereus*. On the other hand, the concentrations of hydrogen peroxide tested by us are several times higher than the concentrations reported to be produced by the organism (40 to 55 μg/ml) (1).

We conclude that *L. acidophilus* inhibits the growth of *C. pylori* in vitro. This inhibitory effect may be related to an extracellular secretory product(s) of *L. acidophilus*, probably lactic acid, and is unrelated to the pH of this product(s). The significance of this finding in the treatment of *C. pylori*-associated acid-peptic disease needs to be evaluated.

ACKNOWLEDGMENT

We thank Dilip R. Karnad for his valuable suggestions during the study and in preparing the manuscript.

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